MINIREVIEW

Pathophysiological Effects of Nicotine on the Pancreas: An Update[1]

PARIMAL CHOWDHURY,[2]* STEWART MACLEOD,† KODETTHOR B. UDUPA,*‡ AND PHILLIP L. RAYFORD*

*Departments of *Physiology and Biophysics, †Surgery, and ‡Geriatrics, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205*

Epidemiological evidence strongly suggests an association between cigarette smoking and pancreatic diseases. It is well recognized that nicotine, a major component in cigarette smoke, is an addictive agent and, therefore, reinforces smoking behavior. The current review update focuses on the genetics of nicotine dependence and its role on the development of pancreatic diseases. The role of smoking and nicotine in pancreatitis and pancreatic cancer development is also discussed. Exposure of laboratory animals to nicotine clearly supports the notion that nicotine can induce pancreatic injury. The mechanism by which nicotine induces such effects is perhaps mediated via signal transduction pathways in the pancreatic acinar cell, leading to enhanced levels of intracellular calcium release, resulting in cytotoxicity and eventual cell death. The induction of pancreatic injury by nicotine may also involve activation and expression of protooncogene, H-*ras*, which can increase cytosolic calcium via second messenger pathways. Development of pancreatic carcinoma in cigarette smokers as observed in human populations may be the result of activation and mutation of the H-*ras* gene. A possible pathogenetic mechanism of nicotine in the pancreas activating multiple signal transduction pathways is schematically summarized in Figure 1.

[Exp Biol Med Vol. 227(7):445–454, 2002]

Key words: nicotine; gastrointestinal function; pancreas; pancreatic injury; mechanism of action

This work was supported, in part, by a grant from the University of Arkansas for Medical Sciences.

[1] This manuscript is an update of a previously published Minireview entitled "Pathophysiological Effects of Nicotine on the Pancreas" (Proc Soc Exp Biol Med 218: 168–173, 1998).

[2] To whom requests for reprints should be addressed at Department of Physiology and Biophysics, Slot #505, University of Arkansas For Medical Sciences, 4301 West Markham Street, Little Rock, Arkansas 72205. E-mail: chowdhuryparimal@uams.edu

1535-3702/02/2277-0445$15.00

In 1998, we published a mini-review on the pathophysiological effects of nicotine on the pancreas (1). Because the deleterious effects of nicotine as they relate to the use of tobacco are continuously being documented, the purpose of this communication is to provide updated information on nicotine and its effects on the pancreas. In addition, we have added information on the genetics of nicotine dependence and have summarized the possible mechanisms by which nicotine may act in the etiology of pancreatic disorders.

Nicotine, a major component of tobacco and cigarette smoke, is an addictive agent and has been characterized as a drug of abuse by the U.S. Surgeon General (2–4). Approximately 430,000 persons die of causes related to smoking cigarettes and approximately 30% of those deaths are due to some form of cancer (5, 6). In France, pancreatic carcinoma is a major health concern, as it kills more than 6000 people each year (7). Cigarette smoking is the major risk factor in the cause of this disease. These phenomena are a worldwide tragedy because according to research studies, a significant number of patients cease to smoke when advised to do so by a physician (8, 9). The economic burden due to abuse of this drug is substantial because of the well-documented pathophysiological effects of nicotine on organs in the cardiovascular, respiratory, hepatic, renal, and nervous systems (4).

The association of nicotine exposure through cigarette smoking with the increased incidence of pancreatitis and pancreatic cancer has been reported (10–24). A survey on the association between cigarette smoking and pancreatic cancer showed that cigarette smokers had a significantly higher risk (70%) of developing pancreatic cancer in comparison with non-smokers (14–22). When compared with non-smokers, subjects who smoke filtered cigarettes had a

50% elevated risk. The proportion of pancreatic cancer attributable to cigarette smoking was 29% in blacks and 26% in whites (16). Most of the data linking cigarette smoke/nicotine to pancreatic diseases were gathered in humans. Studies conducted with animals have shown that nicotine or its metabolites could induce pathological and functional changes in the pancreas (25–31). The current review will present an update and discuss our current understandings of the pathophysiology of the exocrine pancreas induced by nicotine. A possible mechanism of action of nicotine on the induction of pancreatic pathology will be discussed. Before we describe the action of nicotine on pancreas, it appears justifiable to review nicotine dependency and the genetics behind this dependency.

The Genetics of Nicotine Dependence

The use of tobacco products, both cigarettes as well as smokeless tobacco, continues to be a major health problem in the United States. An individual's risk of becoming dependent on nicotine may rely on a complex mix of pharmacological, psychological, and socioeconomic factors. However, recent evidence indicates that genetics may be an important factor in the risk of becoming addicted to nicotine. Studies of tobacco product use among twins (32–34), families, and adopted siblings indicate that tobacco use is influenced by heredity (35–37). The influence of an individual's genetic makeup on their risk of nicotine addiction has only recently been explored.

Genes that are involved in nicotine metabolism, and therefore nicotine tolerance, may influence an individual's risk of becoming nicotine dependent. Individuals who are more efficient metabolizers of nicotine may be less likely to have adverse reactions, such as light-headedness and nausea, upon initial exposure and are therefore more likely to continue using tobacco products. Candidate genes involved in tobacco addiction include CYP2A6 and CYP2D6, which are involved in the metabolism of nicotine to cotinine. Pianezza *et al.* (38) found an under-representation of individuals with low-activity alleles for CYP2A6 among a tobacco-dependent group, indicating that individuals with low CYP2A6 activity were less likely to become dependent on nicotine. They also found that smokers with low activity CYP2A6 alleles smoked significantly fewer cigarettes per week, indicating that lower CYP2A6 activity may be related to lower tolerance to nicotine. More recent studies by Oscarson *et al.* (39–41) and Zabetian *et al.* (42) have cast some doubt concerning these results. Using different genotype methods, these groups have found much lower allele frequencies for low activity CYP2A6 among various populations, which may limit the power of studies using genotypes for CYP2A6.

In addition to a direct effect through activation of tobacco-specific nitrosamines such as 4-(*N*-methyl-*N*-nitrosamino)1-(3-pyridyl)-1-butanone (NNK), CYP2D6 may play a role in lifetime tobacco exposure because it also metabolizes nicotine to cotinine and thereby could increase tolerance to nicotine. Although Ayesh *et al.* (43) reported an association between efficient metabolizers of debrisoquin (catalyzed by CYP2D6) and lung cancer risk, subsequent studies have been inconsistent in identifying an association between CYP2D6, smoking, and lung cancer risk (44). This could be due to the complexity of CYP2D6 metabolism of tobacco products. A recent CYP2D6 genotyping study seems to support earlier reports by identifying an association between efficient metabolizers, lung cancer, and moderate smoking exposure (45).

Polymorphic alleles in the D2 dopamine receptor gene affect the availability of dopamine and have been implicated in individual vulnerability to nicotine dependence among tobacco smokers. Nicotine and other drugs such as alcohol and cocaine induce euphoria in users that is thought to be the result of activation of the mesolimbic dopaminergic reward system of the brain (46, 47). Nicotine activates nicotinic receptors that in turn enhance dopamine release in areas of the brain that are thought to be involved in reward (48–50). The involvement of the dopaminergic system in the reinforcement activity of nicotine may be related to the highly addictive properties of the drug. The human dopamine receptor is polymorphic, with two minor alleles termed the *Taq*IA allele (A_1 and A_2) and the *Taq*IB allele (B_1 and B_2). The functional significance of these polymorphic alleles has been determined using labeled D2 dopamine receptor ligands *in vitro* (51) and *in vivo* (52). These experiments show reduced numbers of D2 dopamine receptors in the brains of individuals who were homozygous or heterozygous for the A_1 allele (A_1A_1 or A_1A_2) compared with those who were homozygous for the more common allele (A_2A_2) at this locus. Because the dopamine receptor D2 (DRD2) is an integral part of the dopaminergic reward system, subjects with reduced numbers of dopamine receptors may compensate for this deficiency by using nicotine to increase brain dopamine levels.

Spitz *et al.* (53), in a case control study of lung cancer patients, found that the B_1B_2 genotype was more common in chronic smokers compared with non-smokers, whether they were cases or controls. Smokers with the least common A_1 or B_1 alleles tended to be younger when they started smoking and attempted to quit smoking fewer times compared with smokers with the more common DRD2 alleles. Noble *et al.* (37) determined that allele frequencies for the A_1 allele were higher in current and former smokers (45.6% and 40.0%, respectively) compared with non-smokers (28.0%). In a similar study, Comings *et al.* (54) found that smokers who were unsuccessful in quitting had an allele frequency of 48.7% for the DRD2 A_1 allele compared with 18.2% for non-smokers. In addition, light smokers had a 37.5% allele frequency for A_1 versus 52.2% for heavy smokers. Taken together, these data suggest that the polymorphic alleles of the DRD2 may be predictive markers for individuals who are at risk of becoming addicted to nicotine.

A functional polymorphism in the promoter region of the serotonin transporter gene (5-hydroxytryptamine transporter or 5-HTT) consists of a 44-bp deletion/insertion that

corresponds to short (s) and long (l) versions of the promoter. The short promoter variant reduces the transcriptional activity of the gene and results in decreased 5-HTT expression (55). Evidence for the function of this polymorphism includes transfection of lymphoblastoid cells with reporter vectors containing the long and short forms of the 5-HTT promoter polymorphism. It was found that the basal and induced activity of the l form was twice that of the s form. In addition, the expression of the native 5-HTT gene in lymphoblastoid cell lines from subjects with different 5-HTT genotypes was found to vary. Cells from l form homozygotes produced 1.4 to 1.7 times as much 5-HTT mRNA compared with homozygotes with the s form of the gene. In addition, $[^3H]$5-HT uptake in cells that were homozygous for the l form of 5-HTT was 1.9–2.2 times that of cells with either the heterozygous or the homozygous s form of the gene. Taken together, these data demonstrate that the s form of the 5-HTT promoter polymorphism is responsible for lower production of 5-HTT and would be expected to produce lower levels of serotonin reuptake in individuals with this form of the gene.

Deficiency in serotonin reuptake may increase the risk of impulsive/aggressive behavior as well as the risk of depression. Neuroticism is a set of personality traits that include anxiety, depression, impulsiveness, and vulnerability factors that have been implicated in the risk of becoming a smoker as well as becoming dependent on nicotine. These traits have also been associated with difficulty in quitting smoking. There is evidence that the 5-HTT promoter polymorphism is related to smoking behavior and to nicotine dependence. Hu *et al.* (56) determined the 5-HTT promoter polymorphism genotype for 759 current, former, and lifelong smokers and found a relationship between smoking behavior, neuroticism, and 5-HTT genotype. Lerman *et al.* (57) also found that smokers who were heterozygous or homozygous for the s allele for 5-HTT were more likely to be dependent on nicotine compared with those who were homozygous for the l allele.

The action of dopamine is terminated by the catabolic action of the enzymes catecholamine-*O*-methyltransferase (COMT) and monoamine oxidase (MAO). Both of these enzymes are polymorphic, and allelic variants of these enzymes that confer different activity on their respective enzymes may contribute to individual differences in susceptibility to substance abuse, including nicotine. A common single nucleotide polymorphism in the COMT gene results in an amino acid change of valine to methionine at residue 108 or 158 in soluble and membrane-bound COMT, respectively. The variants exhibit a 3- to 4-fold variation in COMT activity, with the methionine containing variant having low activity. Higher activity of COMT and/ or MAO could be responsible for lower dopamine levels, and individuals with lower dopamine levels may compensate by using nicotine products.

Future studies designed to explore interindividual differences in nicotine and dopamine metabolism will be useful in identifying individuals who are at increased risk of becoming dependent on nicotine. The results of these studies will also facilitate the development of smoking cessation strategies that are targeted to individual differences in nicotine and dopamine metabolism. Before we discuss the effect of nicotine on the pancreas, a brief description of the anatomy of the pancreas is given below.

Anatomy of the Pancreas

The human pancreas weights about 80 g and has two major sections. The endocrine section makes up approximately 2% of the gland, whereas the exocrine section is about 85% of its total mass (58). Nerves, blood vessels, and other tissues make up the remaining portion of the pancreas. There are four major regions of the pancreas: head, tail, body, and neck; these are named according to their anatomical position in the gland. The fine structures of the pancreas are designated as: (a) acinar units, these cells contain zymogen granules that store pancreatic enzymes; (b) ductle units, these cells store water and bicarbonate; (c) basal lamina, is that portion of the pancreas that interfaces between connective tissue and epithelial cells and is composed of laminin, collagen and fibronectin; and, (d) islet cells. The endocrine pancreas has at least four types of cells: A cells secrete glucagon, B cells secrete insulin, D cells secrete somatostatin, and PP cells secrete pancreatic polypeptide (59). Each of these hormones is involved in the regulation of metabolism. The exocrine pancreas is affected mostly by smoking and probably by alcohol abuse.

Pancreatitis And Pancreatic Cancer: Association with Smoking. An estimated 3 million deaths occur worldwide due to tobacco use (60, 61), and it was shown that the number of deaths from pancreatic cancer was 2.5 times higher in women than in men (60). Besides the demographic factors, cigarette smoking has been suggested as the single most important factor for the development of these diseases (62–65). Several studies showed that patients with chronic pancreatitis had an increased risk of developing pancreatic cancer (66–68). An evaluation of 37,450 patients with unspecified, acute, recurrent, and chronic pancreatitis showed an increased risk of pancreatic cancer development in all sub-cohorts (69). Very few animal models of pancreatitis and pancreatic cancer are available to study the etiology of these diseases; however, studies reported in the hamster model showed recurrent pancreatitis resulting in large number and size of pancreatic tumors (70). More recently, mouse models of exocrine pancreatic cancer have been described (71). These models attempted to address the interaction between a genetic change and the tissue, organ, and whole-animal homeostatic mechanisms that tend to restrict unregulated tissue growth. Evidence from these models suggests that ductal neoplasia may be an important component of exocrine pancreatic cancer progression (71).

Metabolism of Nicotine and Its Effects on the Structural and Functional Changes of the Exocrine Pancreas. Cotinine and nornicotine are natural metabolites of nicotine. Many of the pharmacological effects of

tobacco smoking are due to nicotine (72). Nitrosoamine derivatives of nicotine and other metabolites have been reported to be carcinogenic (73, 74). About 80%–90% of the dose of nicotine consumed can be accounted in human urinary metabolites. It has been reported that nicotine is biotransformed to highly reactive chemicals that covalently modify proteins and DNA (75–77). In rodents, hepatic nicotine metabolism is found to involve cytochrome p450s that catalyze the first step of this pathway (78, 79, 81). Major defective CYP2A6 alleles were recently found in cigarette smokers (80, 40–42).

It has been demonstrated that pancreatitis could be induced in mice fed on a caerulein- and choline-deficient ethionine (CDE) supplemented diet (82–87). The major histopathological changes noted in these animals included cytoplasmic vacuolation, cellular interstitial edema, and cellular necrosis with pyknotic nuclei and karyorrhexis. The appearance of cytoplasmic vacuoles in the exocrine pancreas was considered an early pathological marker of pancreatic injury (87). The vacuoles were found to contain digestive and lysosomal enzymes (88–90), and upon activation, they promote degenerative changes in the pancreas (90, 91). Exposure of animals to nicotine has been shown to induce morphological changes in the pancreas similar to those induced by caerulein and CDE diets (25, 26, 29–31). However, it is not clear whether the changes induced by nicotine also involve activation of proenzymes to active enzymes leading to further tissue destruction.

The precise pathological effects of nicotine and its metabolites on the exocrine pancreas are still obscure. Tobacco smoking, diabetes mellitus, cholelithiasis, and pancreatitis increase the risk of pancreatic cancer (92). Hedberg and colleagues (93) found that a disturbed regulation of pancreatic enzyme secretion, or a regulatory dysfunction of the pancreatic gland, could contribute to the development of pancreatic carcinoma in patients after partial gastrectomy. Their studies suggest that the hormone CCK, which was shown to induce experimental neoplasia, is not involved in this phenomenon. In 30 patients with chronic pancreatitis, it has been reported that postprandial levels of CCK were increased above those found in normal patients (94). There was no difference in basal levels of CCK and in basal and postprandial levels of somatostatin between normal and diseased patients.

We have shown that rats given nicotine in their water ate approximately 10% less food than controls given only water (95). In a separated group of rats given 10% less food than other rats that were allowed to eat food *ad libitum*, rats on less food in each study showed loss of body weight and impaired pancreatic enzyme secretion (96). In a study with humans, Winter and colleagues (97) showed that severe undernourishment resulted in primary gastric and pancreatic secretory dysfunction. In these studies, pancreatic injury was not assessed; however, the studies do suggest that nicotine, by reducing food intake, may play a role in pancreatic injury in humans.

Effects of Nicotine on Gastrointestinal Function. Gastrointestinal secretions in man are affected by cigarette smoking (99). Evidence suggests that nicotine has a direct effect on pancreatic secretions (26–30, 99–102). It has been shown that when rabbits were exposed to nicotine, there was a significant decrease in secretion of duodenal bicarbonate (102). A decreased responsiveness to secretagogues in the pancreas was also found in rats exposed to nicotine (25, 26, 30, 31). Exposure of isolated pancreatic acini to nicotine *in vitro* enhanced secretion of hydrolases and newly synthesized proteins (29). These studies suggest that nicotine and its metabolites have a definitive effect on exocrine pancreatic secretions.

In a study with 207 patients, Heilkius and colleagues (103) concluded that levels of pancreatic enzymes are elevated in a significant proportion of patients with inflammatory bowel disease and with more extensive and active disease. In addition, sclerosing cholangitis also seemed to be associated with increases in pancreatic enzymes. In these patients, urinary amylase levels were higher in smokers than in non-smokers and ex-smokers. Brown (98), in a study with 14 patients, has shown that the smoking of only one cigarette can result in decreased volume and bicarbonate output by the pancreas. He concludes that this effect of nicotine may play a role in the formation of duodenal ulcers in humans.

The studies reported in humans and in our animal studies are consistent in the fact that in both instances, the total content of pancreatic enzymes are elevated in the pancreas with either smoking or nicotine. However, responsiveness of pancreatic enzyme secretion by CCK from isolated acinar cells from animals exposed to nicotine was significantly decreased. The reduced secretion and increase in enzyme content in the pancreas may be a trigger for induced pathogenesis. The dose-response and time course effect of nicotine-induced pathology (e.g., edematous, vacuolar, and pyknotic changes, as well as alterations in mitochondria and other organelles) need to be further examined and characterized.

Chronic cigarette smoking has been directly linked to pulmonary emphysema with correlated reduction in endogenous antiproteases (104). It has been shown that changes in the basal serum levels of the gastrointestinal hormones CCK (105–108), or serum enzymes such as amylase and lipase (109–112), were associated with pancreatic injury (110–113). It is of interest to note that when subjected to a single injection of secretin, there were a significantly higher serum concentrations of pancreatic digestive enzymes in smokers than in non-smoking controls (114), suggesting some form of pancreatic injury occurred in smokers.

Possible Mechanism of Action of Nicotine on the Exocrine Pancreas. Gastrointestinal hormones such as CCK, carbachol, and secretin are ligands for pancreatic receptors and act through specific receptor pathways, mediating complex signal transduction events, resulting in exo-

cytosis of pancreatic enzymes from zymogen granules of the acinar cells (107–109, 115).

A pancreatic acinar cell model as described earlier by many investigators (109, 115–120) demonstrates the mechanism of underlying pathologic changes induced by nicotine (Fig. 1). Acinar cells are programmed to respond to a given stimulus with a coordinated release of secretory granule content. This response indicates the existence of intracellular messengers that in turn transduce the external signal for an increased rate of vesicle membrane fusion and secretory action. The intracellular messengers play a regulatory role in exocytotic secretion and are the key factors in signal transduction pathways (109, 115, 117, 121). Two major classes of receptors were identified in acinar cells based on their response to different agonists: those coupled to mobilization of cellular calcium (CCK, bombesin, and carbachol) and those coupled to activation of adenylate cyclase (secretin and vasoactive intestinal peptide) (115, 121, 122).

A schematic diagram describing the multiple signal transduction pathways in an acinar cell is shown in Figure 1. Preliminary data obtained in our own laboratory suggest that these pathways are directly or indirectly involved in the inhibition of nicotine-induced exocrine pancreatic secretion and retention of enzymes (25–27, 31, 122). As shown in Figure 1 (pathway 1), the binding of CCK and cholinergic agents to their respective receptors results in the release of inositol phosphate and 1, 2-diacylglycerol. In turn, inositol phosphate induces calcium mobilization and activates calmodulin-dependent protein kinases. 1, 2-Diacyl glycerol activates and translocates protein kinase C from the cytosolic to membranous site. Both protein kinase C activation and calcium mobilization are important intermediary steps for pathways of exocrine pancreatic secretion (115, 121).

Data from our investigations suggested that, at least in rats, nicotine induced an inhibition of amylase release, as demonstrated by their responsiveness to CCK and carbachol (31). This observation was associated with an increase in the total cellular amylase content. Furthermore, CCK receptor binding capacity measured in isolated membranes showed no difference between control and nicotine-treated acini (31). These results suggest that postreceptor mechanisms are involved in this altered stimulus-secretion coupling. (A second possible pathway as shown in Figure 1, pathway 2.)

Nicotine is an agonist of the nicotine cholinergic receptor (nAChR) in the central nervous system (CNS) (123). It is widely accepted that it exerts pharmacological effects as a result of interactions with these receptors (123–126). Significant evidence exist in the literature that suggests that nAChRs is the primary site of nicotine action in the CNS (123–126). Several laboratories have demonstrated that prolonged exposure of mice and rats to nicotine and other nicotinic agonists produces a significant increase in the number of agonist binding sites in many brain regions, including cortex, striatum, thalamus, hippocampus, and hypothalamus (125–128). There is also evidence from human *post mortem* studies indicating that cigarette smokers have increased [^{3}H]nicotine binding sites in the brain when compared with non-smokers (129).

It has also been shown that nicotinic receptor activation would result in calcium entry through the open nAChR channels (130, 131), increased calcium influx through voltage-dependent calcium channels (132–134), and increased release of intracellular calcium (135, 136). In bovine adrenal chromaffin cells, it has also been demonstrated that there were two distinct calcium pools that summate within the cell leading to a greater calcium signal (135–137). Com-

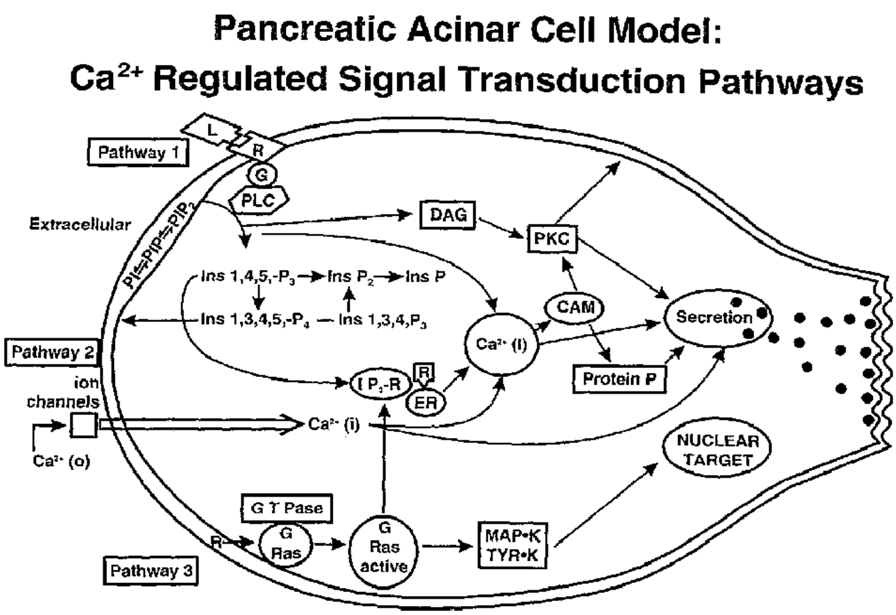

Figure 1. Pancreatic acinar cell model showing induction of multiple Ca^{2+} regulated signal transduction pathways (Printed with permission from Lippincott Williams & Wilkins. Eur J Gastroenterol Hepatol 12(8): 869–877, 2000).

petitive radioligand binding studies conducted with ^{3}H-nicotine in isolated rat pancreatic acinar cells in our laboratory showed no or little binding of nicotine to surface receptors (122). A significant amount of ^{3}H-nicotine remained bound inside the cytoplasmic compartment of the acinar cell (122). Further studies show that CCK and carbachol, which mobilize intracellular calcium, facilitated the increased accumulation of nicotine in isolated pancreatic acinar cells (123), suggesting the involvement of intra- and extracellular calcium as major mediators of nicotine entry into the acinar cells and inducing altered exocrine pancreatic secretion.

Various investigators have also demonstrated that nicotine stimulation of adrenal chromaffin cells led to an increase in the concentration of inositol trisphosphate (InsP3) (138), InsP4, and InsP5 (139), as well as enhanced translocation of protein kinase C (140) from the cytosol to the membranes. These effects are calcium dependent and can be mimicked by stimulation of the cells with a depolarizing concentration of potassium. Indeed, increases in intracellular calcium, promoting cytotoxicity due to nicotine, IP_3, and other agonists in various cellular systems including pancreatic acinar cells, have been reported (141, 142).

It appears important to examine the relationship between nicotine, intra- and extracellular calcium pools, and intracellular signaling paths. Data from our laboratory suggest that the entry of nicotine into the acinar cell is perhaps regulated by Ca^{2+} receptor pathways (122). Intracellular signals such as inositol phosphates, protein kinases, diacyl glycerol, and activation of G proteins can lead to calcium release and mobilization. Therefore, future studies may be directed to ascertain whether nicotine affects these pathways (see pathways 1, 2, and 3 in Fig. 1). (A third possible pathway of altered exocrine pancreatic pathology may be due to overexpression of oncogenes by nicotine as shown in pathway 3, Fig. 1.)

Regulatory genes can be used to study the various signals for tissue specificity and differential expression of the gene products (143–145). The pattern of pancreatic gene expression is extensively modified during pancreatitis (146, 147). The proto-oncogenes are overexpressed in embryonic tissues (148–150), in the pancreas during carcinogenesis, after induction of growth by mitogens, and during regeneration following pancreatectomy (151). Studies in rats exposed to nicotine via inhalation for 21 days showed the enhancement of expression of a mutant ras p21 protein and activation of the H-*ras* gene in the acinar cells of the pancreas (152).

Mutations in the *ras* gene alter the normal function of the ras gene product, p21 protein, which functions as a signal switch molecule. Altered p21 protein also affects the GTPase-activating protein, which mediates the signal transducing effect of p21 (153–155), thereby inactivating the signaling switch. Thus, the H-*ras* gene-mediated signal transduction pathway might be one of the mechanistic sites by which nicotine induces pancreatic injury. Activation of the *ras* gene product triggers the release of inositol phosphates through receptor-mediated G-protein coupling (153, 154), and also stimulates phospholipase C (PLC) generating "second messengers" such as DAG and IP_3. Consequently, IP_3 stimulates the release of intracellular calcium from endoplasmic reticulum (ER), elevating intracellular Ca^{+2} and predisposes cellular injury (156, 157). Thus, the effects of calcium mobilization via H-ras and IP_3 in response to nicotine may play an important role in enhancing cell damage in the acinar cells (138, 156, 158).

The proteins encoded by the *ras* gene are essential for the transduction of diverse extracellular signals to intracellular targets (159–162). The *ras* proteins bind guanine nucleotides with high affinity, and cycle between an active GTP-bound state and an inactive guanosine diphosphate (GDP)-bound state (163, 164). The *ras* proteins regulate a key point in signal transduction pathways between the mitogenic growth factors and ultimately the nuclear transcription factors that regulate cell division (165–168).

Thus, the activation of these multiple signal transduction pathways due to nicotine exposure results in high levels of intracellular calcium release and may be responsible for cell cytotoxicity and cell injury. Future studies to explore the relationship of nicotine on gene expression and mutation and with cancer development in the pancreas are also warranted.

1. Chowdhury P, Rayford PL, Chang LW. Pathophysiological effects of nicotine On the pancreas. Proc Soc Exp Biol Med **218**:168–173, 1998.
2. Miller, NS. Nicotine addiction as a disease In: Cocores JA, Ed. The Clinical Management of Nicotine Dependence. New York: Springer-Verlag, pp66–78, 1991.
3. Surgeon General. The Health Consequences of Using Smokeless Tobacco. USPHS, Bethesda, MD, NIH Publication #86-2874, 1986.
4. U.S. Public Health Service: Smoking and health: A report of the Surgeon General, Department of Health, Education and Welfare, Public Health Service, Office of the Assistant Secretary for Health, Office of Smoking and Health (DHEW Publication No. PHS79-50066). Washington, D.C.: U.S. Government Printing Office, pp13–41, 1979.
5. Okuyemi KS, Harris J, Ahuwalia JS, Wallace DD. Documentation of smoking: role of age, gender and ethnicity. J Assoc Minor Acad Physicians **12**:125–128, 2001.
6. USDHHS. Healthy People: 2000 Review. Washington, D.C.: Government Printing Press, 1994.
7. Caudry M, Bonnel C. Adenocarcinoma of the exocrine pancreas: management and therapeutic hopes. Rev Med Intern **20**:810–815, 1999.
8. USDHHS. Smoking Cessation: Clinical Practice Guideline: Rockville, MD: Public Health Service, Agency for Health Care Policy and Research, 1996.
9. Ross G, Colwell L. Randomized controlled trial of smoking advice: final 20 year results. J Epidemiol Commun Health **46**:75–77, 1992.
10. Fernandez E, La Vecchia C, Porta M, Negri E, d'Avanzo B, Boyle P. Pancreatitis and the risk of pancreatic cancer. Pancreas **11**:185–189, 1995.
11. Wynder EL, Mabuchi K, Maruchi N, Fortner JG. Epidemology of cancer of the Pancreas. J Natl Canc Inst **50**:645–667, 1973.
12. Gold EB, Cameron JL. Chronic pancreatitis and the risk of pancreatic cancer. N Engl J Med **323**:1485–1486, 1993.
13. Lowenfels AB, Maisonneuve P, Cavalline G, Ammann RW, Lankisch PG, Anderson JR, DiMagno EP, Andren-Sanberg A, Domillof

A. The International Pancreatic Study Group. Pancreatitis and risk of pancreatic cancer. N Engl J Med **328**:1433–1437, 1993.
14. Mack TM, Yu Mc, Hanisch R, Henderson BE. Pancreas cancer and smoking, beverage consumption and past medical history. J Natl Cancer Inst **76**:49–60, 1986.
15. Farrow DC, Davis S. Risk of pancreatic cancer in relation to medical history and use of tobacco, alcohol and coffee. Int J Cancer **45**:816–820, 1990.
16. Silverman DT, Dunn JA, Hoover RN, Schiffman M, Lillmoe KD, Schoenberg JB, Brown LM, Greenberg RS, Hayes RB, Swanson GM, Wacholder S, Swartz AG, Liff JM, Pattern LM. Cigarette smoking and pancreas cancer: a case control study based on direct interviews. J Natl Cancer Inst **86**:1510–1516, 1986.
17. Miller BA, Silverman DT, Kaplan R. Pancreas. In: Miller BA, Ries LA, Hanky BF, et al., Eds. Cancer Statistics Review: 1973-1990. Bethesda, MD: NIH Publication #93-2789, NCI, 1993.
18. Olsen, GW, Mandel JS, Gibson RW, Wattenburg LW, Schuman LM. A case control study of the pancreatic cancer and cigarettes, alcohol coffee and diet. Am J Public Health **79**:1016–1019, 1989.
19. Bueono de Mesquita HB, Maisonneuve P, Moerman CJ, Runia S, Boyle P. Life time history of smoking and exocrine carcinoma of the pancreas: population based case control study in the Netherlands. Int J Cancer **49**:846–822, 1991.
20. Zatonski WA, Boyle P, Przewozniak K, Maisonneuve P, Drosik K, Walker AM. Cigarette smoking, alcohol, tea and coffee consumption and pancreas cancer risk: a case control study from Opole, Poland. Int J Cancer **53**:601–607, 1993
21. Howe GR, Jain M, Burch JD, Miller AB. Cigarette smoking and cancer of the pancreas: evidence from a population based case control study in Toronto, Canada. Int J Cancer **47**:323–328, 1991.
22. U.S. Dept Health and Human Services. The Health Benefits of Smoking Cessation: A Report of Surgeon General, 1990. DHHS Publication No. (CDC) 90-8416. Washington, D.C.: CDC PHS DHHS, 1990.
23. Rivenson A, Hoffman D, Prokopczyk B, Amin S, Hecht SS. Induction of lung and exocrine pancreas tumors in F344 rats by tobacco specific any areca derived *N*-nitrosamines. Cancer Res **48**:6912–6917, 1988.
24. Pour PM, Rivenson A. Induction of a mixed ductal-squamous-islet cell carcinoma in a rat treated with a tobacco-specific carcinogen. Am J Pathol **134**:627–631, 1989.
25. Chowdhury P, Rayford PL, Chang LW. Inductions of pancreatic acinar cell pathology via inhalation of nicotine. Proc Soc Exp Biol Med **20**:159–164, 1992.
26. Chowdhury P, Hosotani R, Chang LW, Rayford PL. Metabolic and pathologic effects of nicotine on the gastrointestinal tract and pancreas of rats. Pancreas **5**:222–229, 1990.
27. Chowdhury P, Ami M, Hosotani R, Rayford PL. Meal stimulated exocrine pancreatic secretion and release of GI peptides in normal and nicotine treated rats. Regul Peptides **33**:11–20, 1991.
28. Dubick MA, Palmer R, Lau PP, Morrill RR, Geokas MC. Altered exocrine pancreatic function in rats treated with nicotine. Toxicol Appl Pharmacol **96**:132–139, 1988.
29. Majumder APN, Davis GA, Dubick MA, Geokas MC. Nicotine stimulation of protein secretion from isolated rat pancreatic acini. Am J Physiol **248**:G158–G163, 1985.
30. Lau PP, Dubick MA, Yu GS, Morill PR, Geokas MC. Dynamic changes of pancreatic structure and function of rats treated chronically with nicotine. Toxicol Appl Pharmacol **104**:457–465, 1990.
31. Chowdhury P, Doi R, Tangoku A, Rayford PL. Structure and functional changes of rat exocrine pancreas exposed to nicotine. Int J Pancreatol **18**:257–264, 1995.
32. Cheng LS, Swan GE, Carmelli DA. Genetic analysis of smoking behavior in family members of older adult males. Addiction **95**:427–435, 2000.
33. Carmelli D, Swan GE, Robinett D, Fabsitz R. Genetic influence on smoking—a study of male twins [see comments]. N Engl J Med **327**:829–833, 1992.
34. Swan GE, Carmelli D, Cardon LR. The consumption of tobacco, alcohol, and coffee in Caucasian male twins: a multivariate genetic analysis. J Substance Abuse **8**:19–31, 1996.
35. Noble EP, St. Jeor ST, Hughes GR. Genetics of smoking: a brief review. Behavior Ther **17**:335–345, 1986.
36. Noble EP, Ritchie T, Syndulko K, St. Jeor ST, Fitch RJ, Brunner RL, Sparkes RS. D2 dopamine receptor gene and cigarette smoking: a reward gene? Medical Hypotheses **42**:257–260, 1994.
37. Noble EP. The DRD2 gene, smoking, and lung cancer [editorial; comment]. J Natl Cancer Inst **90**:343–345, 1998.
38. Pianezza ML, Sellers EM, Tyndale RF. Nicotine metabolism defect reduces smoking [letter]. Nature **393**:750, 1998.
39. Oscarson M, Gullsten, Rautio A, Bernal ML, Sinues B, Dahl ML, Stengard JH, Pelkonen O, Raunio H, Ingelman-Sundberg M. Genotyping of human cytochrome P450 2A6 (CYP2A6), a nicotine C-oxidase. FEBS Lett **438**:201–205, 1998.
40. Oscarson M, McLellan RA, Gullsten H, Agundez J A, Benitez J, Rautio A, Raunio H, Pelkonen O, Ingelman-Sundberg M. Identification and characterisation of novel polymorphisms in the CYP2A locus: implications for nicotine metabolism. FEBS Lett **460**:321–327, 1999.
41. Oscarson M, McLellan RA, Gullsten H, Yue QY, Lang MA, Bernal ML, Sinues B, Hirvonen A, Raunio H, Pelkonen O, Ingelman-Sundberg M. Characterization and PCR-based detection of a CYP2A6 gene deletion found at a high frequency in a Chinese population. FEBS Lett **448**:105–110, 1999.
42. Zabetian CP, Gelernter J, Cubells JF. Functional variants at CYP2A6: new genotyping methods, population genetics, and relevance to studies of tobacco dependence. Am J Medical Genet **96**:638–645, 2000.
43. Ayesh R, Idle JR, Ritchie JC, Crothers MJ, Hetzel MR. Metabolic oxidation phenotypes as markers for susceptibility to lung cancer. Nature **312**:169–170, 1984.
44. Christensen PM, Gotzsche PC, Brosen K. The sparteine/debrisoquine (CYP2D6) oxidation polymorphism and the risk of lung cancer: a meta-analysis. Eur J Clin Pharmacol **51**:389–393, 1997.
45. Legrand-Andreoletti M, Stucker I, Marez D, Galais P, Cosme J, Sabbagh N, Spire C, Cenee S, Lafitte JJ, Beaune P, Broly F. Cytochrome P450 CYP2D6 gene polymorphism and lung cancer susceptibility in Caucasians. Pharmacogenetics **8**:7–14, 1998.
46. Wise RA, Rompre PP. Brain dopamine and reward. Ann Rev Psychol **40**:191–225, 1989.
47. Koob GF. Drugs of abuse: anatomy, pharmacology and function of reward pathways. Trends Pharmacol Sci **13**:177–184, 1992.
48. Brazell MP, Mitchell SN, Joseph MH, Gray JA. Acute administration of nicotine increases the in vivo extracellular levels of dopamine, 3,4-dihydroxyphenylacetic acid and ascorbic acid preferentially in the nucleus accumbens of the rat: comparison with caudate-putamen. Neuropharmacology **29**:1177–1185, 1990.
49. Pontieri FE, Tanda G, Orzi F, Di Chiara, G. Effects of nicotine on the nucleus accumbens and similarity to those of addictive drugs [see comments]. Nature **382**:255–257, 1996.
50. Pontieri FE, Passarelli F, Calo L, Caronti B. Functional correlates of nicotine administration: similarity with drugs of abuse. J Mol Med **76**:193–201, 1998.
51. Noble EP, Blum K, Ritchie T, Montgomery A, Sheridan PJ. Allelic association of the D2 dopamine receptor gene with receptor-binding characteristics in alcoholism. Archi Gen Psych **48**:648–654, 1991.
52. Pohjalainen T, Rinnie JO, Nagren K, Lehikoinen P, Anttila K, Syvalahti EK, Hietala J. The A1 allele if the human D2 receptor gene predicts low D2 receptor availability in healthy volunteers. Mol Psych **3**:256–260, 1998.
53. Spitz MR, Shi H, Yang F, Hudmon K S, Jiang H, Chamberlain RM, Amos C I, Wan Y, Cinciripini P, Hong WK, Wu X. Case-control study of the D2 dopamine receptor gene and smoking status in lung cancer patients [see comments]. J Natl Cancer Inst **90**:358–363, 1998.
54. Comings DE, Ferry L, Bradshaw-Robinson S, Burchette R, Chiu C, Muhleman D. The dopamine D2 receptor (DRD2) gene: a genetic risk factor in smoking. Pharmacogenetics **6**:73–79, 1996.
55. Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Muller CR, Hamer DH, Murphy DL. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region [see comments]. Science **274**:1527–1531, 1996.
56. Hu S, Brody CL, Fisher C, Gunzerath L, Nelson ML, Sabol SZ, Sirota LA, Marcus SE, Greenberg BD, Murphy DL, Hamer DH. Interaction between the serotonin transporter gene and neuroticism in cigarette smoking behavior. Mol Psych **5**:181–188, 2000.
57. Lerman C, Caporaso NE, Audrain J, Main D, Boyd NR, Shields PG. Interacting effects of the serotonin transporter gene and neuroticism

in smoking practices and nicotine dependence. Mol Psych 5:189–192, 2000.
58. Brockman D. Development of the pancreas. In: Berger HG, Warshaw AL, Buchler MW, Car-Locke DLNeoptolemos JP, Russell C, Sarr MG, Eds. The Pancreas. Oxford, UK: Blackwell Science Vol 1:pp3–10, 1988.
59. Nauck MA. Physiology and pathophysiology of endocrine pancreatic secretion. In: Berger HG, Warshaw AL, Buchler MW, Car-Locke DL, Neoptolemos JP, Russell C, Sarr MG, Eds. The Pancreas. Oxford, UK: Blackwell Science Publishers, Vol 1:pp101–137, 1988.
60. Tobacco Research Implementation Group, National Cancer Institute, National Institutes of Health. Tobacco Research Implementation Plan, Priorities for Tobacco Research Beyond the Year 2000, November [Monograph]. Bethesda, MD, 1998.
61. Weiderpass E, Partanen T, Kaaks R, Vaunio H, Porta M, Kauppinen Y, Ojajarvi A, Bofetta P, Malats N. Occurrence, trends and environment etiology of pancreatic cancer. Scand J Work Environ Health 24:165–174, 1998.
62. Gold EB, Goldin SB. Epidemiology of and risk factors for pancreatic cancer. Surg Oncol Clin North Am 7:67–91, 1998.
63. Niderhuber JE, Brennon MF, Menck HR. The National Cancer Data Base Report on Pancreatic Cancer. Communication from the American College of Surgeons Commission on Cancer and the American Cancer Society. Cancer 76:1671–1677, 1995.
64. Hirayama T. Epidemiology of pancreatic cancer in Japan. Jpn J Clin Oncol 19:208–215, 1989.
65. Shibata A, Mack TM, Paganini-Hill A, Ross RK, Henderson BE. A prospective study of pancreatic cancer in the elderly. Int J Cancer 58:46–49, 1994.
66. Cavallini G, Talamini G, Vaona B, Bovo P, Fillippini M, Rigo L, Angelini G, Vantini I, Riela A, Frulloni L. Effect of alcohol and smoking on pancreatic lithogenesis in the course of chronic pancreatitis. Pancreas 9:42–46, 1994.
67. Talamini G, Bassi C, Falconi M, Frulloni L, Di Francesco B, Vaona B, Bovo P, Rigo L, Castagini A, Angelini G, Vantini I, Pederzoli P, Cavallini G. Cigarette smoking: an independent risk factor in alcoholic pancreatitis. Pancreas 12:131–137, 1996.
68. Lowenfels AB, Maisonneuve P, Cavallini G, Ammann RW, Lankisch PG, Anderson JR, Dimagno EP, Andren-Sandberg A, Domellof L. Pancreatitis and the risk of pancreatic cancer. International Pancreatic Study Group. N Engl J Med 328:1433–1437, 1993.
69. Karlson BM, Ekbom A, Josefsson S, McLaughlin JK, Fraumeni JF, Nyren O. The risk of pancreatic cancer following pancreatitis: an association due to confounding? Gastroenterology 113:587–592, 1997.
70. Pour PM, Takahashi M, Donnelly T, Stepan K. Modification of pancreatic carcinogenesis in hamster model. IX. Effect of pancreatitis. J Natl Cancer Inst 71:607–613, 1983.
71. Sandgreen EP. Mouse models of exocrine pancreatic cancer. In: Pancreatic Cancer: From Genes to Treatment. Baltimore, MD: John Hopkins University School of Medicine Conference Proceedings (June 2001 Scientific Meeting of the Lustgarten Foundation for Pancreatic Cancer Research), June 14, p20, 2001.
72. Cashman JR, Park SB, Yang ZC, Wrighton SA, Jacob P III, Benowitz N. Metabolism of nicotine by human liver microsomes: stereoselective formation of trans-nicotine N′-oxide. Chem Res Toxicol 5:639–646, 1992.
73. Hoffmann D, Hecht SS. Nicotine derived *N*-nitrosoamines and tobacco related cancer: current status and future directions. Cancer Res 45:935–944, 1985.
74. Hecht SS, Hoffmann D. Tobacco specific nitrosoamines, an important group of carcinogens in tobacco and tobacco smoke. Carcinogenesis 9:875–884, 1988.
75. Carmella SG, Kagan SS, Hecht SS. Evidence that a hemoglobin adduct of 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone is a 4-(3-pyridyl)-4-oxibutyl carboxylic acid ester. Chem Res Toxicol 5:76–80, 1992.
76. Shigenega MK, Trevor AJ, Castangoli N Jr. Metabolism-dependent covalent binding of (S)-(5-3H) nicotine to liver and lung microsomal macromolecules. Drug Metab Disposition 16:397–402, 1988.
77. Belinsky SA, White CM, Baucheron JA, Richardson FC, Swenberg JA, Anderson M. Accumulation and persistence of DNA adducts in respiratory tissue of rat following multiple administration of the tobacco-specific carcinogen 4-(*N*-methyl-*N*-nitrosoamino)-1-(3-pyridyl)-1-butanone. Cancer Res 46:1280–1284, 1986.
78. Hammond DK, Bjercke RJ, Langone JJ, Strobel HW. Metabolism of nicotine by rat liver cytochromes P-450: assessment utilizing monoclonal antibodies to nicotine and cotinine. Drug Metabolism Disposition 19:804–808, 1991.
79. Nakayama H, Okuda H, Nakashima T, Imaoka S, Funae Y. Nicotine metabolism by rat hepatic cytochrome P450 S. Biochem Pharmacol 45:2554–2556, 1993.
80. Oscarson M, Gullsten H, Rautio A, Bernal ML, Sinues B, Dahl ML, Stengard JH, Pelkonen O, Raunio H, Ingelman-Sundberg M. Genotyping of human cytochrome P 450 2A6 (CYP2A6), a nicotine C-oxidase. FEBS Lett 438:201–205, 1998.
81. Hanioka N, Jinno H, Kitazawa K, Tanaka-Kagawa T, Nishimura T, Ando M, Ogawa K. In vitro biotransformation of atrazine by rat liver microsomal cytochrome p 450 enzymes. Chem Biol Interact 116:181–198,1998.
82. Lombardi B, Estes LW, Longnecker DS. Acute hemorrhagic pancreatitis c massive necrosis) with fat necrosis induced in mice by DL-ethionine with a choline-deficient diet. Am J Pathol 79:464–480, 1975.
83. Niederau C, Ferrell LD, Grendell JH. Caerulein induced acute necrotizing pancreatitis in mice: protective effects of proglumide, benzotript and secretin. Gastroenterology 88:1192–1204, 1985.
84. Watanabe I, Baccino FM, Steer ML, Meldolesi J. Supromaximal caerulein stimulation and ultrastructure of rat pancreatic acinar cells early morphological alterations during development of experimental pancreatitis. Am J Physiol 246:G457–G467, 1984.
85. Koike K, Steer ML, Meldolesi J. Pancreatic effects of ethionine, blockade of exocytosis, and appearance of crinophagy and autophagy preceding cellular necrosis. Am J Physiol 242:G297–G307, 1982.
86. Adler G, Hahn C, Kern HF, Rao KN. Caerulein induced pancreatitis in rats: increased lysosomal enzyme activity and autophagocytosis. Digestion 32:10–18, 1985.
87. Niederau C, Grendall JH. Intracellular vacuoles in experimental acute pancreatitis in rats and mice are an acidified compartment. J Clin Invest 81:229–236, 1988.
88. Rao K, Zuretti N, Raccino M, Lombardi B. Acute hemorrhaegic pancreatitis in mice. The activity of lysosomal enzymes in the pancreas and liver. Am J Pathol 98:45–59, 1980.
89. Niederau C, VanDyke RW, Scharschmidt BF, Grendell JH. Rat pancreatic zymogen granules: an actively acidified compartment. Gastroenterology 91:1433–1442, 1986.
90. Steer ML, Meldolesi J, Figarella C. Pancreatitis: the role of lysosomes. Dig Dis Sci 29:934–938, 1984.
91. Yamaguchi H, Kimura T, Mimura K, Nawata H. Activation of proteases in caerulein-induced pancreatitis. Pancreas 4:565–571, 1989.
92. Kalapothaki V, Tzonou A, Hsieh CC, Toupadaki N, Karakatsani A, Trichopoulos D. Tobacco, ethanol,coffee, pancreatits, diabetes mellitus, and cholelithiasis as risk factors for pancreatic carcinoma. Cancer Causes Control 4:375–382, 1993.
93. Hedberg M, Janzon L, Rehfeld JF, Borgstrom A. Long-term effects on the regulation of pancreatic secretion after gastric surgery. Dig Surg 16:111–116, 1999.
94. al-Eryani S, Duris I, Payer J, Huorka M, Kratochvilova H, Ondrejka P. Plasma cholecystokinin and somatostatin levels in chronic pancreatitis patients. Hepatogastroenterology 33:869–874, 2000.
95. Chowdhury P, Doi R, Tangoku A, and Rayford PL. Structural and functional changes of rat pancreas exposed to nicotine. Int J Pancreatol 18:257–264, 1995.
96. Chowdhury P, Rayford PL. Effect of food restriction on plasma cholecystokinin levels and exocrine pancreatic function in rats. Ann of Clin Lab Sci 31:376–382, 2001
97. Winter TA, Marks T, Callanan M, O'Keefe SJ, Bridger S. Impaired pancreatic secretion in severly malnourished patients is a consequence of primary pancreatic dysfunction. Nutrition 17:230–235, 2000.
98. Brown P. The influence of smoking on pancreatic function in man. Med J Aust 2:290–293, 1976.
99. Murthy SNS, Dinoso VP Jr., Clearfield HR, Chey WY. Simultaneous measurements of basal, pancreatic, gastric acid secretion, plasma gastrin and secretin during smoking. Gastroenterology 73:758–761, 1977.
100. Konturek SJ, Solomon TE, McGreight WG, Johnson LR, Jacobson

WD. Effect of nicotine on gastrointestinal secretions. Gastroenterology 13:361–365, 1972.
101. Bynum TE, Solomon TE, Johnson LR, Jacobson ED. Inhibition of pancreatic secretion in man by cigarette smoking. Gut 13:361–365, 1972.
102. Solomon TE, Solomon N, Shanbour LL, Jacobson ED. Direct and indirect effects of nicotine on rabbit pancreatic secretion. Gastroenterology 67:276–283, 1974.
103. Heikius B, Neimela S, Lehtola J, Karttunen TJ. Elevated pancreatic enzymes in inflammatory bowel disease are associated with extensive disease. Am J Gastroenterol 94:1062–1069, 1999.
104. Chowdhury P, Bone RC, Louria DB, Rayford PL. Effect of cigarette smoke on human trypsin inhibitory capacity and antitrypsin concentrations Am Rev Resp Dis 126:177–179, 1982.
105. Richter JM, May RJ. Disorders of the pancreas. In: Chopra S, May RJ, eds. Pathology of the Gastrointestinal Diseases. Boston: Little Brown & Co, pp529–573, 1990.
106. Bloom SR. Clinical Syndromes of gut hormones. In: Thompson JC, ed. Gastrointestinal Endocrinology, Receptors, and Postreceptor Mechanisms. New York: Academic Press, pp479–490, 1990.
107. Kontuerek SJ. Gastrointestinal hormones and gastric secretion. In: Jerzy GB, ed. Gastrointestinal Hormones. New York: Raven Press, pp529–564, 1990.
108. Chey WY. Gastrointestinal Hormones, pancreatic biliary and intestinal secretins. In: In: Jerzy GB, ed. Gastrointestinal Hormones. New York: Raven Press, pp565–586, 1980.
109. Williams JA, Korc M, Dormer RL. Action of secretagogues on a new preparation of functionally intact isolated pancreatic acini. Am J Physiol 235:E517–E524, 1978.
110. Schmidt J. Lewandrowskik, Fernandez-del Castillo, Mandaielli U, Compton CE, Warshaw AL, Rattner DW. Histopathologic correlates of serum amylase activity in acute experimental pancreatitis Dig Dis Sci 37:1426–1433, 1992.
111. Agarwal N. Pitchumoni, Sivaprasad AV. Evaluating tests for acute pancreatitis. Am J Gastroenterol 85:356–366, 1990.
112. Nordestgaard AG, Wilson SE,Williams RA. Correlation of serum amylase levels with pancreatic pathology and pancreatitis etiology. Pancreas 3:159–161, 1988.
113. Dubick MA, Mar G, Mayer AD, MaJumder APN, McMahon MJ, Geokas MC. Digestive enzymes and protease inhibitors in plasma from patients with acute pancreatitis. Pancreas 2:187–194, 1987.
114. Andriulli A, Masoero G, Amato A, Felder M, Benitti V, Dobrilla G, De la Pierre M, Verme G. Serum immunoreactive cationic trypsinogen response to secretin in normal subjects. Am J Gastroentrol 78:579–583, 1983.
115. Williams JA, Hootman SR. Stimulus-secretion coupling in pancreatic acinar cells. In: Gro VLW, Brooks FB, Dimagno EP, et al., Eds. Exocrine Pancreas: Biology, Pathology and Diseases. New York: Raven Press, pp123–139, 1986.
116. Besterman HS, Adrian TE, Bloom SR, Christofides ND, Mallinson CN, Ponti V, Lombardo L, Modigliani R Guerin S, South M. Pancreatic and gastrointestinal hormones in chronic pancreatitis. Digestion 24:195–208, 1982.
117. Bruzzone R. The molecular basis of enzyme secretion. Gastroenterology 99:1157–1176, 1990.
118. Doi R, Chowdhury P, Rayford PL. Agonist-regulated alteration of the affinity of pancreatic muscarinic cholinergic receptors. J Biol Chem 268:22436–22443, 1993.
119. Brannon PM. Primary cultures of pancreatic acinar cells. In: Thompson JC, Ed. Gastrointestinal Endocrinology Receptors and Post-Receptor Mechanisms. New York: Academic Press, Vol 16:pp199–209, 1990.
120. Brannon PM, Orisson DM, Kretchmer N. Primary cultures of rat pancreatic acinar cells in serum free medium. In Vitro Cell Dev Biol 21:6–14, 1985.
121. Neer EJ. Heterotrimeric G Proteins: organizer of transmembrane signals. Cell 80:249–257, 1995.
122. Chowdhury P, Doi R, Nishikawa M, Takaori K, Rayford PL. Carbachol and cholecystokinin enhance accumulation of nicotine in rat pancreatic acinar cells. Pancreas 10:154–160, 1995.
123. Hillard CJ. Neurochemistry of nicotine. In: Watson RR, Ed. Drugs of Abuse and Neurobiology. Boca Raton, FL: CRC Press, pp85–114, 1992.
124. Wonnacott S. The paradox of nicotine acetylcholine receptor up regulation by nicotine. Trends Pharmacol Sci 11:216–219, 1990.
125. Schwartz RD, Keller KJ. In vivo regulation of [3H]acetylcholine recognition sites in brain by nicotinic cholinergic drugs. J Neurochem 45:427–433, 1985.
126. Schwartz RD, Keller KJ. Nicotine cholinergic receptor binding sites in the brain: regulation in vivo. Science 220:214–216, 1983.
127. Marks MJ, Burch JB, Collins AC. Effects of chronic nicotine infusion on tolerance development and nicotinic receptors. J Pharmacol Exp Ther 226:817–825, 1983.
128. Marks MJ, Stitzel JA, Collins A. Time course study of the effects of chronic nicotine infusion on drug response and brain receptors. J Pharmacol Exp Ther 235:619–628, 1985.
129. Benwell ME, Belfour DJK, Anderson JM. Evidence that tobacco smoking increases the density of (-)-[3H]nicotine binding sites in human brain. J Neurochem 50:1243–1247, 1988.
130. Miledi R, Parker I, Schalow G. Transmitter induced calcium entry across post synaptic membrane of frog end plates measured using arsenazo III. J Physiol 300:197–212, 1980.
131. Wada A, Tadara H, Izumi F, Kobayashi H, Yanihara N. Influx of 22Na through acetylcholine receptor associated Na channels: relationship between 22Na influx, 45Ca influx and secretion of catecholamines in cultured bovine adrenal medulla cells. Neuroscience 15:283–292, 1985.
132. Cena V, Nicholas GP, Sanchez-Garcia P, Kirpekar SM, Garcia AG. Pharmacological dissection of receptor associated and voltage sensitive ionic channels involved in catecholamine release. Neuroscience 10:1455–1462, 1983.
133. Boarder M, Marriott D, Adams M. Stimulus secretion coupling in cultured chromaffin cell dependency on external sodium and on dihydropyridine sensitive calcium channels. Biochem Pharmacol 36:163–167, 1987.
134. Hillard CJ. Effects of nicotine and nicotine agonist on calcium influx into brain synaptosomes. NIDA Mongraph Serv 81:324, 1988.
135. Burgoyne RD. The relationship between secretion and intracellular free calcium in bovine adrenal chromaffin cells. Biosci Rep 4:605–611, 1984.
136. Eberhard DA, Holz RW. Cholinergic stimulation on inositol monophosphate formation in bovine adrenal chromaffin cells: distinct nicotinic and muscarinic mechanisms. J Neurochem 49:1634–1643, 1987.
137. Firestone JA, Browning MD. Calcium signalling in bovine adrenal chromaffin cells: additive effects of histamine and nicotine. Synapse 17:268–274, 1994.
138. Nakaki T, Sasakawa N, Yamamoto S, Kato R. Functional shift for muscarinic to nicotinic cholinergic receptors involved in inositol triphosphate and cyclic GMP accumulation during primary culture of adrenal chromaffin cells. Biochem J 251:397–403, 1988.
139. Sasakawa N, Nakaki T, Kato R. Rapid increase in inositol penkisphosphate accumulation by nicotine in cultured adrenal chromaffin cells. FEBS Lett 261:378–380, 1990.
140. TerBush DR, Bittner MA, Holt RW. Ca^{2+} influx causes rapid translocation of protein kinase C to membranes. J Biol Chem 262:18873–18879, 1988.
141. Travali S, Koniecki J, Petralia S, Baserga R. Oncogenes in growth and development. FASEB J 4:3209–3214, 1990.
142. Zhang GH, Melvin JE. Nicotine increases $[Ca^{2+}]_i$ in rat sublingual mucous acini by stimulating neurotransmitter release from presynaptic terminals. Proc Soc Exp Biol Med 207:292–301, 1994.
143. Hillard CJ, Graf WK.. Studies of the effect of nicotine on synaptosomal calcium accumulation using Fura-2. NIDA Res Monograph 165:331–332, 1990.
144. Hunter T. Cooperation between oncogenes. Cell 64:249–270, 1991.
145. Cowley B, Chadwick L, Grantham J, Calvert J. Sequential protooncogene expression in regenerating kidney following acute renal injury. J Biol Chem 264:8389–8393, 1989.
146. Iovanna JL, Lechene de La Porte P, Dagorn JC. Expression of genes associated with dedifferentiation and cell proliferation during pancreatic regenertion following acute pancreatitis. Pancreas 7:712–718, 1992.
147. Iovanna JL, Keim V, Michel R, Dagorn JC. Pancreatic gene expression is altered during acute experimental pancreatitis in the rat. Am J Phyisol 261:485–489, 1991.
148. Slaman D, Cline M. Expression of cellular oncogenes during embry-

onic and fetal development of the mouse. Proc Natl Acad Sci U S A **81**:7141–7145, 1984.
149. Zimmerman K, Yancopoulos G, Collum R, Smith RK, Kohl NE, Denis KA, Nan MM, Witte ON, Toran-Allerand D, Gee CE, Minna JD, Alt FW. Differential expression of myc family genes during marine development. Nature **319**:780–783, 1986.
150. Muller R, Slamon D, Tremblay J, Cline M, Verma I. Differential expression of cellular oncogenes during pre- and postnatal development of the mouse. Nature **299**:640–644, 1982.
151. Calvo E, Dussetti N, Cadenas MB, Dagorn JC, Iovanna JL. Changes in gene expression during pancreatic regeneration: activation of c-myc and H-ras oncogenes in the rat pancreas. Pancreas **6**:150–156, 1991.
152. Chowdhury P, Montague DC, Rayford PL, Chang LW, Lyn-Cook B-D. Nicotine alters pancreatic gene (Ha-ras) expression and induces point mutation and exocrine pancreatic injury. XVI Proceedings of the International Cancer Congress. New Delhi, India. Rao RS, Deo MG, Sanghvi LD, Mitra I, eds. Bologna, Italy: Monduzzi Editoire, pp369–373, 1994.
153. Alonso T, Srivastava S, Santos E. Alterations of G-protein coupling function in phosphoinositide signaling pathways of cells transformed by ras and other membrane-associated and cytoplasmic oncogenes. Mol Cell Biol **10**:3117–3124, 1990.
154. Gibbs JB, Marshall MS, Scolnick EM, Dixon RAF, Vogel US. Modulation of guanine nucleotides bound to ras in NIH3T3 cells by oncogenes, growth factors, and the GTPase activating protein (GAP). J Biol Chem **265**(33):20437–20442, 1990.
155. Valencia A, Kjeldgaard M, Pai EF, Sander C. GTPase domains of ras p21 oncgene protein and elongation factor Tu: analysis of three-dimensional structures, sequence families, and functional sites. Proc Natl Acad Sci U S A **88**:5443–5447, 1991.
156. Schanne FA, Kane AB, Young EE, Farber JL. Calcium dependence of toxic cell death: a final common pathway. Science **206**:700–702, 1979.
157. Chien KR, Farber JL. Microsomal membrane dysfunction in ischemic liver cells. Arch Biochem Biophys **180**:191–198, 1997.
158. Majumdar APN, Vesenka GD, Dubick MA, Geokas MC. Evaluation of the role of calcium in cytotoxic injury in isolated rat pancreatic acini. Biochem Biophys Res Commun **139**:530–537, 1986.
159. Haubruck H, McCormick F. Ras p21: effects and regulation. Biochem Biophys Acta **1072**:215–229, 1991.
160. Marx J. Two major signal pathways linked. Science **262**:988–990, 1993.
161. Mulachy LS, Smith MR, Stacey DW. Requirements for ras protooncogene function during serum-stimulated growth of NIH 3T3 cells. Nature **313**:241–243, 1985.
162. Beitel GJ, Clark SG, Horvitz HR. *Caehorhabditis elegans* ras gen let-60 acts as a switch in the pathway of vulval induction. Nature **348**:503–509,1990.
163. Simon MA, Bowtell DD, Dodson GS, Laverty TR, Rubin GM. Ras 1 and a putative guanine nucleotide exchange factor perform crucial steps in signaling by the sevenless protein tyrosine kinase. Cell **67**:701–716, 1991.
164. Bourne HR, Sanders DA, McCormick F. The GTPase superfamily: a conserved switch for diverse cell functions. Nature **348**:125–132, 1990.
165. Capella G, Cronauer-Mitra S, Peinado MA, Perucho M. Frequency and spectrum of mutations at condons 12 and 13 of the c-K-ras gene in human tumors. Environ Health Persp **93**:125–131, 1991.
166. Barbacid M. Ras gene. Annu Rev Biochem **56**:779–827, 1987.
167. Marx J. Forging a path to the nucleus. Science **260**:1588–1590, 1993.
168. Lowy DR, Zhang K, DeClue JE, Williamsen BM. Regulation of p21 ras activity. Trends Genet **7**:346–351, 1991.